A FRET approach to phosgene detection†

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A FRET approach towards potential detection of phosgene is presented, which is based on a selective chemical reaction between phosgene (or triphosgene as a simulant) and donor and acceptor fluorophores.

Phosgene ($C(O)Cl_2$) is a highly toxic and chemically reactive gas that has been used as a chemical warfare agent.[1] It is a lung irritant and a very insidious poison, which does not irritate immediately, even when fatal concentrations are inhaled. Inhalation of phosgene causes severe lung injury, the full effects appearing several hours after exposure. Phosgene first came into prominence during World War I, when it was used, either alone or mixed with chlorine, against troops. There is a renewed interest in the development of phosgene sensors due to the increased threat of weapons deployment by terrorists. Sensing methods for phosgene are typically electrochemical or based on remote spectroscopic detection techniques.[2] At the same time, optical sensors for this gas are very rare.[3] This is surprising since reactive chromophores and fluorophores are now actively used for sensing other chemical agents.[4] Such sensors utilize a change of absorption or fluorescence upon detection and better address the needs of specialists in that they are simple, fast and reliable. In this communication, we present our preliminary studies towards the design of potential phosgene sensors based on fluorescence resonance energy transfer (FRET).[5] It includes a selective chemical reaction between phosgene and donor and acceptor fluorophores, which bring them together, within the appropriate Förster distance (Fig. 1). Phosgene serves here as a cross-linking agent. When the emission spectrum of the donor overlaps with the absorption spectrum of the acceptor, FRET occurs upon irradiation of the donor and strong emission of the acceptor can be detected. This reports on the presence of phosgene.

For this project, triphosgene ($CCl_3OC(O)OCCl_3$) was used as a phosgene simulant. It is less toxic and easier to handle. In organic chemistry, triphosgene has been utilized as a safe phosgene replacement for making ureas.[6] To take advantage of this chemical property, commercially available laser dyes[7] coumarin 2 and coumarin 343 were functionalized with primary amino groups (see ESI†). Taken separately, thus prepared coumarins **1** and **2** smoothly react with triphosgene at room temperature in $CHCl_3$ in the presence of triethylamine (TEA) with the formation of the corresponding ureas (see ESI†). Donor coumarin **1** absorbs at λ = 343 nm and emits at λ = 425 nm in $CHCl_3$. When light with the latter wavelength is used for excitation of acceptor coumarin **2**, it strongly emits at λ = 468 nm (in $CHCl_3$).

When both coumarins **1** and **2** are mixed together with triphosgene in the presence of TEA in $CHCl_3$, hybrid urea **3** forms as well, in a statistical yield (Fig. 2). It was isolated on a preparative scale and fully characterized. In molecule **3**, the donor and acceptor units are covalently linked and positioned within ~20 Å of one another. The absorption spectrum shows two strong bands at λ_{max} = 343 and 438 nm that belong to the coumarin 2 and coumarin 343 units, respectively. More important however is that at low concentrations of $\leqslant 10^{-5}$ M, the donor excitation at λ = 343 nm leads to the acceptor emission at λ = 464 nm. In a control experiment only utilizing acceptor coumarin **2**, no emission in this region was detected under these conditions.‡ This implies a FRET phenomenon in urea **3**.

The phosgene sensing experiment involves the formation of urea **3** and its fluorescence. Coumarins **1** and **2** were mixed in a 1 : 1 ratio at the concentration range between 5×10^{-4} and 10^{-2} M in $CHCl_3$, TEA (~10 equiv.) was added and then triphosgene was introduced. Addition of TEA prevents protonation of the amino groups and does not affect the fluorescence. Aliquots were taken and diluted to 10^{-6} M,‡ and the emission was recorded upon excitation at λ = 343 nm. Significant fluorescence enhancement at λ = 464 nm was detected (Fig. 3). This is particularly important since the acceptor unit alone does not emit under these conditions. The fluorescence increase is obviously due to the formation of urea

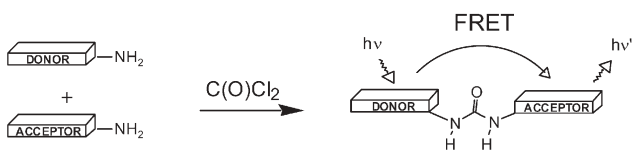

Fig. 1 A FRET based approach to the detection of phosgene.

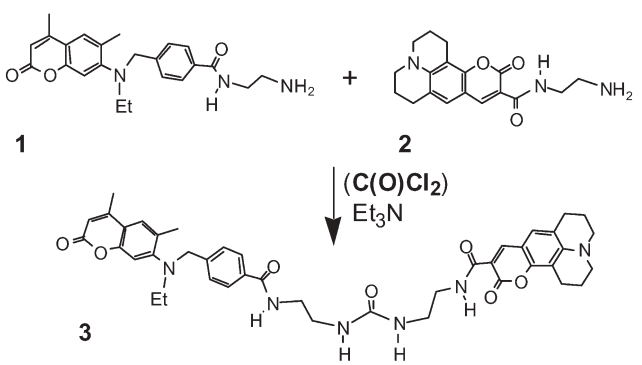

Fig. 2 Coumarins **1** and **2** smoothly and rapidly react with phosgene/triphosgene with the formation of urea **3**.

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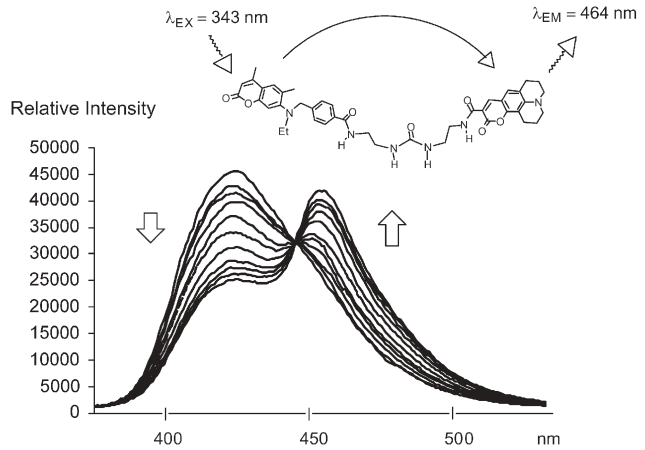

Fig. 3 Typical changes in fluorescence emission spectra upon titrations with triphosgene. Coumarins **1** and **2** were mixed in a 1 : 1 ratio at 10^{-3} M in $CHCl_3$, TEA (10 equiv.) was added and then triphosgene (0.03 to 5 equiv.) was introduced. Aliquots were taken and diluted to 10^{-6} M, and the emission was recorded upon excitation at λ = 343 nm. The arrows indicate the fluorescence changes upon increasing the triphosgene concentration.

Fig. 4 The "naked eye" detection of triphosgene. Left: coumarins **1** and **2** in $CHCl_3$ before addition of triphosgene. Right: after addition of ~5 equiv. of triphosgene. The experiments were performed at 10^{-3} M, and the solutions were then diluted to 10^{-6} M. Both vials are irradiated at λ = 365 nm with a laboratory UV lamp.

3.§ Simultaneously, the fluorescence from the donor unit at λ = 424 nm decreased. This is due to the quenching, indicating that efficient energy transfer took place from the donor to the acceptor. The fluorescence changes were clearly seen already upon addition of as little as ~0.1 equiv. of phosgene (recalculated from 0.03 equiv. triphosgene), which places the detection limit for this particular FRET system at 5×10^{-5} M.

In addition to fluorescence spectroscopy, the response of compounds **1** and **2** to triphosgene can be conveniently followed even with the naked eye (Fig. 4). The increased fluorescence intensity was clearly observed within seconds after the addition of triphosgene.

In conclusion, a proof-of-principle is presented on how to detect phosgene utilizing it as a cross-linking agent in a designed FRET system. This systems is selective, since other gases/agents rarely can serve for cross-linking.¶ The sensor design is clearly not limited to coumarins: the dyes do not react with phosgene but rather report on its presence. In principle, any other FRET acceptors and donors can be used. Further work will be directed towards lowering the detection limits.

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Notes and references

‡ At 10^{-4} M and higher concentrations, excitation at λ = 343 nm leads to the increased fluorescence of acceptor coumarin **2**.

§ The formation of urea **3** under these conditions was confirmed by ^{1}H NMR spectroscopy; at 10^{-5} M and lower the reaction is extremely slow. In addition to **3**, two other, symmetrical ureas are also formed with two donor or two acceptor fragments. The acceptor–acceptor urea does not emit fluorescence upon excitation at λ = 343 nm at these concentrations.

¶ One possible exception could be thionyl chloride ($S(O)Cl_2$). These data will be reported elsewhere.

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